

Reemergence of Lymphocytic Choriomeningitis Mammarenavirus, Germany

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Lymphocytic choriomeningitis mammarenavirus (LCMV) is a globally distributed zoonotic pathogen transmitted by house mice (*Mus musculus*). We report the reemergence of LCMV (lineages I and II) in wild house mice (*Mus musculus domesticus*) and LCMV lineage I in a diseased golden lion tamarin (*Leontopithecus rosalia*) from a zoo in Germany.

Lymphocytic choriomeningitis mammarenavirus (LCMV) is an enveloped virus with a bisegmented genome of single-stranded, ambisense RNA (1). The small (S) segment of $\approx 3,400$ nt encodes structural components, including the glycoprotein (GP) and nucleocapsid protein (NP), whereas the large (L) segment of $\approx 7,200$ nt encodes the L (RNA polymerase) and Z proteins (1,2). First identified in St. Louis, Missouri, USA, in 1933 (3), LCMV is a zoonotic pathogen transmitted through contact with excreta and secretions of infected house mice (*Mus musculus*), the reservoir host (4). More recently, LCMV RNA was also detected in wild wood mice (*Apodemus sylvaticus*) from Spain (5).

In humans, LCMV can cause symptoms ranging from influenza-like illness to meningitis and encephalitis (6). Infection during pregnancy may lead to neurologic and developmental problems in infants

(7). New World primates (family Callitrichidae) are also susceptible to infection, resulting in callitrichid hepatitis, a lethal infection exhibiting histopathologic lesions in the brain, liver, and lymphoid tissues (8).

During 1968–1973, a total of 48 human cases of LCMV infection were reported in Germany, many of which originated from pet hamsters (*Mesocricetus auratus*) (6,7,9). Thereafter, 6 prenatal or postnatal infections were reported during 1991–1997 in Germany, most of which were believed to have originated from pet rodents (10). During 1999–2000, a total of 4 callitrichid hepatitis cases were reported in Germany: 1 in a Goeldi's monkey (*Callimico goeldii*) and 3 in pygmy marmosets (*Cebuella pygmaea*) (8).

Very little is known on the distribution and prevalence of LCMV in mice from Germany, the most comprehensive study being from Ackermann et al. (11) in 1964, which only surveyed the western federal states of Germany (former West Germany). That study found the highest prevalence among house mice in North Rhine-Westphalia, in the western part of West Germany. Most recently, Fornůšková et al. (12) screened nearly 800 mice from the Czech Republic and eastern Germany sampled during 2008–2019 but detected LCMV-positive mice only in the Czech Republic.

Four lineages of LCMV are recognized (I–IV). Lineages I and II are the most common sequences worldwide. Lineage III consists of a single strain from Georgia, USA. Only S-segment sequences exist for lineage IV, entirely comprising sequences obtained from wood mice (*Apodemus sylvaticus*) from Spain. Fornůšková et al. (12) recently proposed that LCMV lineages I and II are host-specific, whereby lineage I is harbored by the house mouse subspecies *M. m. domesticus* and lineage II by the subspecies *M. m. musculus*. This hypothesis is of particular importance in Europe because *M. m. domesticus* and *M. m. musculus* meet

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DOI: <https://doi.org/10.3201/eid2903.221822>

in the house mouse hybrid zone, a $\approx 2,500$ -km-long stretch from Scandinavia to the Black Sea. The hybrid zone acts as a barrier to gene flow and the spread of pathogens between the subspecies (12,13), and, as a consequence, *M. m. domesticus* populations in Germany would be expected to harbor only lineage I.

Although house mice are strongly associated with human settlements, LCMV surveillance in wild

mice in Europe is lacking. This study examines the re-emergence of LCMV in a golden lion tamarin (*Leontopithecus rosalia*; family Callitrichidae) and wild house mice (*M. m. domesticus*) from a zoo in Germany.

The Study

In late 2021, an adult golden lion tamarin from a zoo in western Germany died (Appendix, [Phylogenetic tree showing the relationships between LCMV sequences. The tree is rooted with the Lunk virus \(AB693151\) as an outgroup. The sequences are grouped into four lineages \(I-IV\) based on the classification by Albarrino et al. \(2\). The sequences are listed on the right, with their GenBank accession numbers, strain names, host species, and country of origin. The tree is constructed using Bayesian inference, with bootstrap values indicated at the nodes. A scale bar of 0.4 is shown at the bottom.

Sequences \(from top to bottom\):

- AB627954_WE_Human_JP
- AF004519_Human_US
- FJ607021_Hamster_US
- FJ607026_Hamster_US
- FJ607024_Douglas4707_Human_US
- FJ607025_WE_Human_US
- AB627955_OQ28_Mm_JP
- KT731537_Mmd_GF
- FJ607022_811316_Human_US
- DQ868488_Traub_Mm_US
- FJ607027_810362_Human_US
- JF912084_HP65_2009_1_Mmd_FR
- KM882857_Makokou_Mmd_GA
- MG554170_JX4_Tick_CN
- MG554171_JX14_Tick_CN
- MG554172_JX31_Tick_CN
- FJ607023_810885_Mm_US
- DQ868486_Pasteur_FR
- EU195889_MX_Human_SK
- DQ286932_Mmd_FR
- JN872494_Human_ES
- AY847351_Armstrong_53b
- DQ868484_CH_5692_Cebuella_DE
- OP958779_KS22_089_Mmd_DE
- FJ607019_810366_Human_US
- MZ558313_Mmm_CZ
- MZ558312_Mmm_CZ
- EU136039_Dandenong_YU_to_AU
- OP958777_L_rosalia_DE
- OP958781_KS22_098_Mmd_DE
- AB477530_M1_Mmm_AU
- AB627956_BRC_Mmm_AU
- GQ862981_Bulgaria_Mm_BG
- FJ607020_810935_Human_US
- AB693151_Lunk_virus

Lineages \(I-IV\) are indicated by Roman numerals on the right side of the tree.](https://ww-</p>
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Figure. Phylogeny of the L protein encoding nucleotide sequences of lymphocytic choriomeningitis virus (LCMV) identified in Germany (blue arrows) and reference sequences, constructed by using Bayesian inference. Lunk virus from *Mus minutoides* mice was used as an outgroup. Sequence names are comprised of the GenBank accession number, strain name, host species and country of origin (wherever known). Countries are represented by their International Organization for Standardization code (AU, Australia; BG, Bulgaria; CN, China; DE, Germany; ES, Spain; FR, France; GA, Gabon; GF, French Guiana; JP, Japan; SK, Slovakia; US, USA; YU, former Yugoslavia). Roman numerals (I–IV) represent the different LCMV lineages defined according to Albarrino et al. (2); WE and Armstrong refer to laboratory strains of LCMV. Mm, *Mus musculus*; Mmm, *Mus musculus musculus*; Mmd, *Mus musculus domesticus*.

wnc.cdc.gov/EID/article/29/3/22-1822-App1.pdf). On the basis of the symptoms and an initial diagnosis by the Hessian State Laboratory (Landeslabor Hessen, Giessen, Germany), we conducted further screening for LCMV. The Hessian State Laboratory and the zoo sent tissue samples from the golden lion tamarin and wild mice from the zoo (taken in 2009, 2021, and 2022) to the Friedrich-Loeffler-Institut (Greifswald-Insel Riems, Germany) for molecular and epidemiologic investigations. We extracted and screened nucleic acids for LCMV using conventional reverse transcription PCR (14). We detected LCMV RNA in the brain of the golden lion tamarin and in the kidneys of 55% of wild house mice (*M. m. domesticus*) from 2021 and 2022 (n = 53) but not in any of the house mice from 2009 (n = 82). On the basis of ≈340 nt sequences from the L segment of the virus (GenBank accession nos. OP938541–68), we selected 2 mice with the most dissimilar LCMV sequences and, together with brain tissue from the diseased golden lion tamarin, used them for high-throughput sequencing of complete genomes (GenBank accession nos. OP958777–82) (Appendix).

We used the new complete coding-region sequences (L, GP, and NP) together with all published LCMV genomes to reconstruct phylogenetic trees using the general time reversible substitution model with invariable sites and gamma distribution (MrBayes 3.2.7, <https://nbisweden.github.io/MrBayes>) (15). LCMV sequences of the full coding regions of the L, NP, and GP proteins were almost identical between the golden lion tamarin and 1 of the mice, forming a monophyletic clade within LCMV lineage II (Figure; Appendix Figure). The sequences obtained from the other mouse fell within lineage I (Figure; Appendix Figure). According to the L segment sequences (≈340 nt) obtained from the remaining 27 LCMV-positive mice, both lineages were nearly equally represented in the zoo population (lineage I for 16 mice, lineage II for 11 mice).

We obtained sequences from the mitochondrial DNA d-loop of all LCMV-positive mice and several LCMV-negative mice from 2009 (n = 32), 2021 (n = 12), and 2022 (n = 41). All those sequences identified exclusively the house mouse subspecies *M. m. domesticus* (data not shown).

Conclusions

The high similarity between LCMV lineage II in a golden lion tamarin and a wild house mouse indicates that the virus was passed between wild and captive animals in the zoo. The large number of LCMV lineage I and II strains in the wild house

mouse population at this site suggests either an outbreak after recent introduction from 2 different sources or long-term persistence in the local house mouse population but with very low prevalence in 2009.

Despite considerable effort by researchers to detect LCMV in Germany, the virus remains mostly elusive. Although the route through which LCMV entered the zoo is not known, this event most likely occurred after 2009. The virus may have been brought in through naturally occurring wild animals in the region (e.g., wild house mice) or, although unlikely, through infected zoo animals.

We provide evidence for LCMV lineage II in Germany within an area naturally occupied only by the *M. m. domesticus* subspecies of house mice. The occurrence of both LCMV lineages I and II in *M. m. domesticus* mice does not support the subspecies host specificity proposed by Fornůsková et al. (1). Further evaluation of LCMV association with house mouse subspecies in Germany and other parts of the world will help clarify potential expanded risk to animal and human health.

Acknowledgments

We thank Hartmut Egdmann and zookeepers for collecting the rodents; Patrick Schuhmacher, Dörte Kaufmann, Edyta Janik-Karpińska, Viola Haring, Stephan Drewes, Maria Justiniano Suarez, and Marieke de Cock for help with dissections; and Lukas Wessler, Patrick Zitzow, and Florian Schröder for technical assistance.

The investigations were funded through German Center for Infection Research, Thematic Translational Unit “Emerging Infections” (grant no. 01.808_00).

About the Author

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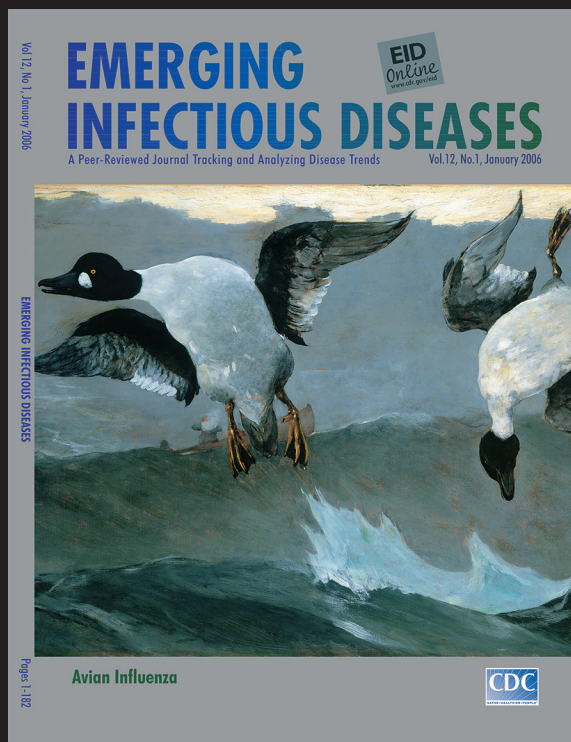
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Appendix

Methods

Pathology and Epidemiology

In late 2021, an adult golden lion tamarin (*Leontopithecus rosalia*) from a German zoo was found unresponsive and later succumbed to illness. A subsequent necropsy of the monkey at the Landeslabor Hessen revealed localized necrotizing hepatitis, low-grade lymphocytic meningitis, interstitial nephritis and interstitial non-inflammatory pneumonia with localized haemorrhages. Tissues were collected and the initial diagnosis of LCMV was given. Tissue samples were then sent to the Friedrich-Loeffler-Institut (FLI) for further investigation.

To determine whether wild house mice (*Mus musculus*) were harboring and potentially spreading LCMV, mice - collected during routine pest management from sites throughout the zoo (2021–2022) - were frozen at -20°C and sent to the FLI for investigation. Furthermore, mice collected from the same zoo in 2009, stored at -20°C , were also sent to the FLI for investigation. The mice were then thawed at 4°C and dissected under Biosafety level 3 conditions.

Nucleic Acid Analyses

Nucleic acids were isolated from homogenized brain, liver or kidney tissue using NucleoMag® VET (Macherey-Nagel, Düren, Germany), per kit instructions, on a KingFisher Flex Purification System (Thermo Fisher Scientific, Waltham, MA, USA). A one-step RT-PCR was performed using SuperScript III RT-PCR Kit (Qiagen, Hilden, Germany) and arenavirus primers, as described in Vieth et al. (1). The products were then resolved by agarose gel electrophoresis (~340 nt segment). BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, MA, USA) was then used to amplify DNA, which was purified using NucleoSEQ® Columns (Macherey-Nagel, Düren, Germany), and used for Sanger sequencing.

By comparing partial sequences (obtained as above, uploaded to GenBank with accession numbers OP938541 – OP938568) from the mice to that from the golden lion tamarin, a mouse with the most similar and a mouse with the most dissimilar sequences were selected for detailed investigation via high-throughput sequencing (HTS). Brain tissue from the golden lion tamarin and kidney tissue from the two mice were used for HTS. Library preparation and HTS was done as detailed described by Pfaff et al. 2022 (2). The taxonomic identification tool RIEMS (3) was used for initial detection of potential pathogens contained in the datasets. Full coding sequences of the LCMV L- and S-segments from the golden lion tamarin (accession numbers OP958777 and OP958778, respectively) and both mice (accession numbers OP958779 - OP958782) were then obtained using de novo assembly with the Genome Sequencer software suite (version 2.6; Roche).

Mice were identified to species level using cytochrome *b* mitochondrial DNA sequences, as described in (4). Subspecies differentiation was done based on mitochondrial d-loop sequence analysis as described previously by Linnenbrink et al. 2013 (5).

Phylogenetic Analyses

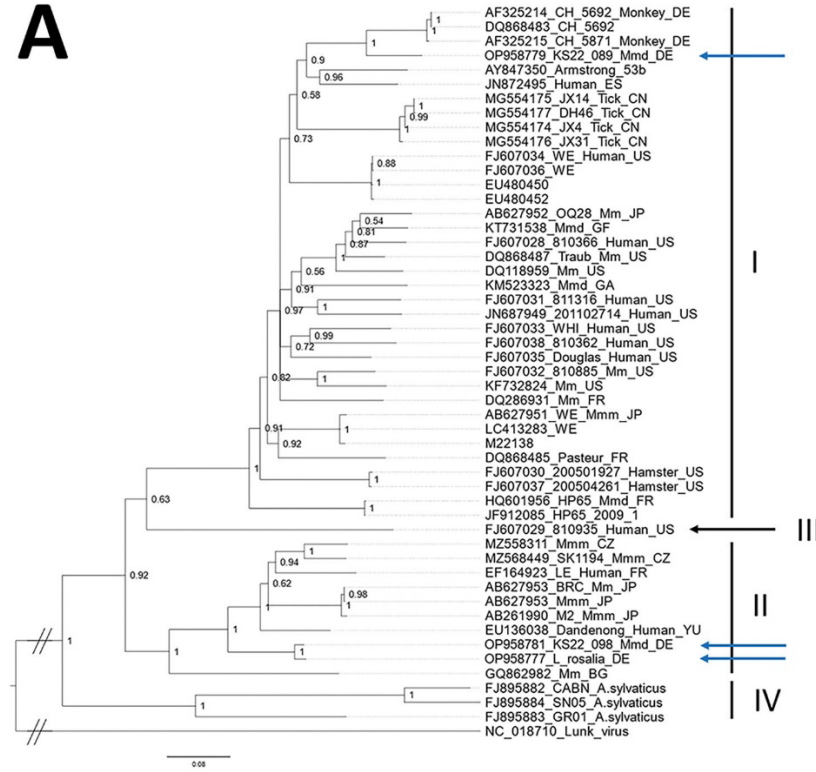
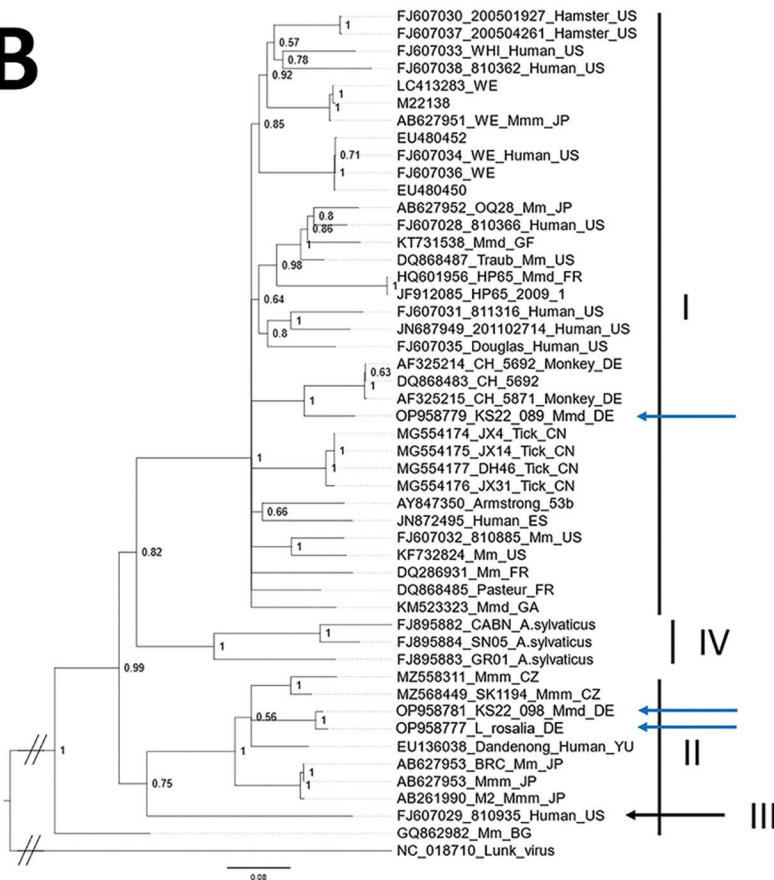
Sequences were aligned in BioEdit (6) using the ClustalW algorithm, and identified using BLAST (NCBI (7)). For the ~340 nt L-segment sequences obtained from the mice, a neighbor-joining tree (Mega11 (8)) was constructed to identify clades within all L-segment sequences.

For the full coding region sequences obtained via HTS, JModelTest2 (9,10) was used to determine the best-fit nucleotide substitution model. The best-fit model for all genes (L, GP and NP) was general time reversible (GTR) with a proportion of invariable sites and gamma distribution. Phylogenetic trees were obtained using Bayesian inference in MrBayes v.3.2.7 (11). Twenty million generations were run with trees sampled every 100 generations. The first 25% were discarded as burn-in.

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A**B**

Appendix Figure. Phylogeny of the nucleotide sequences of lymphocytic choriomeningitis virus (LCMV) encoding (A) glycoprotein and (B) nucleoprotein identified in Germany (blue arrows) and reference sequences, constructed by using Bayesian inference. Lunk virus from *Mus minutoides* mice was used as an outgroup. Sequence names are comprised of, if known, the GenBank accession number, strain name, host species and country of origin. Countries are represented by their ISO code. Roman numerals (I-IV) represent the different virus lineages as defined according to Albariño et al. 2010 (1). “WE” and “Armstrong” are laboratory strains of LCMV. Mm = *Mus musculus*, Mmm = *Mus musculus musculus*, Mmd = *Mus musculus domesticus*, AU = Australia, BG = Bulgaria, CN = China, CZ = Czech Republic, DE = Germany, ES = Spain, FR = France, GA = Gabon, GF = French Guiana, JP = Japan, SK = Slovakia, U.S. = USA, YU = former Yugoslavia.